

UNITED STATES PATENT APPLICATION

For

**SYNTHESIS AND METHODS OF USE OF TETRAHYDROINDOLONE  
ANALOGUES AND DERIVATIVES**

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# SYNTHESIS AND METHODS OF USE OF TETRAHYDROINDOLONE ANALOGUES AND DERIVATIVES

## BACKGROUND OF THE INVENTION

**[0001]** 1. *Field of the Invention:*

**[0002]** The present invention is directed to tetrahydroindolone derivatives and analogues, particularly tetrahydroindolone derivatives or analogues in which the tetrahydroindolone derivative or analogue is covalently linked to another moiety to form a bifunctional conjugate.

**[0003]** 2. *General Background and State of the Art:*

**[0004]** Diseases and degenerative conditions of the central nervous system are among the most severe, long-lasting, and chronic diseases and conditions affecting man. Although much research has been done on such diseases and conditions, effective treatment remains elusive. These diseases and conditions include Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (Lou Gehrig's disease), Parkinson's disease, multiple sclerosis, stroke, and other neurodegenerative disorders, which may be genetic, spontaneous or drug-induced.

**[0005]** There is therefore a need for improved compounds and methods for treating such conditions. The need for such improved compounds and methods has been increased by the discovery that such compounds are capable of increasing neuronal function, stimulating nerve growth or regeneration and can act through the induction of neurotrophic factors such as nerve growth factor, NT-3, brain-derived neurotrophic factor (BDNF), or ciliary neurotrophic factor (CNTF). Such compounds may stimulate nerve regeneration or neurogenesis in the peripheral nervous system or central nervous system, or neuroprotection, and may therefore be of use in the treatment of the diseases and conditions referred to above.

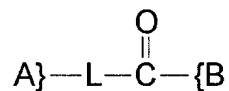
**[0006]** There is therefore a particular need for the development of additional compounds that have improved activity in stimulating neuronal function, regeneration, neurogenesis, and that have neuroprotective activity. There is further a need for compounds that have activities that provide treatment for or relief from symptoms of

diseases and conditions such as Alzheimer's disease, Huntington's disease, Parkinson's disease, multiple sclerosis, stroke and other neurodegenerative disorders, which may be genetic, spontaneous or drug-induced. Examples of these symptoms include reduced cognition, emotional control, and sensory or motor function. There is a particular need for the development of new compounds that have improved bioavailability. There is a further need for compounds with a greater degree of activity as measured by a dose-response curve assay and for compounds with a different spectrum of activities.

**[0007]** One potential basis for such compounds is the bicyclic compound tetrahydroindolone, which is isosteric with purines. However, compounds that incorporate tetrahydroindolone or its analogues and that have activities such as nootropic activity or neuroproliferative activity have not been prepared.

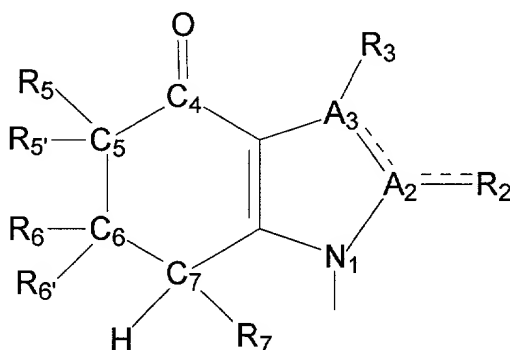
#### INVENTION SUMMARY

**[0008]** One aspect of the present invention is bifunctional conjugates. In general, a bifunctional conjugate according to the present invention has the schematic structure:



where:

(1) A is a 9-atom bicyclic moiety in which the five-membered ring has 1 to 3 nitrogen atoms, the bicyclic moiety having the structure:



where:

(a) N<sub>1</sub> is bonded to L;

(b)  $A_2$  and  $A_3$  are C or N:

(i) if  $A_2$  and  $A_3$  are both C and the bond between  $A_2$  and  $A_3$  is a single bond, then the bond between  $A_2$  and  $R_2$  is two single bonds to two hydrogen atoms or is a double bond in which  $R_2$  is O or S and  $R_3$  is two hydrogen atoms;

(ii) if  $A_2$  and  $A_3$  are both C and the bond between  $A_2$  and  $A_3$  is a double bond, then  $R_3$  is hydrogen, the bond between  $A_2$  and  $R_2$  is a single bond and  $R_2$  is hydrogen, halo, alkyl, alkenyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, or heteroaralkenyl;

(iii) if  $A_2$  and  $A_3$  are both N, then the bond between  $A_2$  and  $A_3$  is a double bond and  $R_2$  and  $R_3$  are not present;

(iv) if  $A_2$  is N and  $A_3$  is C, then the bond between  $A_2$  and  $A_3$  is a double bond,  $R_2$  is not present, and  $R_3$  is hydrogen;

(v) if  $A_2$  is C,  $A_3$  is N, and the bond between  $A_2$  and  $A_3$  is a double bond, then  $R_3$  is not present, the bond between  $A_2$  and  $R_2$  is a single bond, and  $R_2$  is hydrogen, halo, alkyl, alkenyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, or heteroaralkenyl;

(vi) if  $A_2$  is C,  $A_3$  is N, and the bond between  $A_2$  and  $A_3$  is a single bond, then  $R_3$  is hydrogen, alkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl, the bond between  $A_2$  and  $R_2$  is a double bond, and  $A_2$  is O or S;

(c)  $R_5$  is hydrogen, alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl,  $NH_2$ ,  $NHQ_1$ ,  $NQ_1Q_2$ , OH,  $OQ_1$ , or  $SQ_1$ , where  $Q_1$  and  $Q_2$  are alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, heteroaroyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, or heteroaralkylsulfonyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S, and when  $Q_1$  and  $Q_2$  are present together and are alkyl, they can be taken together to form a 5 or 6 member ring which may contain 1 other heteroatom which can be N, O, or S, of which the N may be further substituted with  $Y_2$ , where  $Y_2$  is alkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, heteroaralkylsulfonyl,

alkoxycarbonyl, aryloxy carbonyl, heteroaryloxy carbonyl, aralkoxycarbonyl, heteroaralkoxycarbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl, aralkylaminocarbonyl, or heteroaralkylaminocarbonyl, in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S;

(d)  $R_{5'}$  is hydrogen unless  $R_5$  is alkyl, in which case  $R_{5'}$  is hydrogen or the same alkyl as  $R_5$ ;

(e)  $R_5$  and  $R_{5'}$  can be taken together as a double bond to  $C_5$  and can be O, S,  $NQ_3$ , or C which can be substituted with one or two groups  $R_5$ , where  $Q_3$  is alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, or heteroaroyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S;

(f)  $R_6$  is hydrogen, alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl,  $NH_2$ ,  $NHQ_4$ ,  $NQ_4Q_5$ , OH,  $OQ_4$ , or  $SQ_4$ , where  $Q_4$  and  $Q_5$  are alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, heteroaroyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, or heteroaralkylsulfonyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S, and when  $Q_1$  and  $Q_2$  are present together and are alkyl, they can be taken together to form a 5 or 6 member ring which may contain 1 other heteroatom which can be N, O, or S, of which the N may be further substituted with  $Y_2$ , where  $Y_2$  is alkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, heteroaralkylsulfonyl, alkoxycarbonyl, aryloxy carbonyl, heteroaryloxy carbonyl, aralkoxycarbonyl, heteroaralkoxycarbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl, aralkylaminocarbonyl, or heteroaralkylaminocarbonyl, in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S;

(g)  $R_{6'}$  is hydrogen unless  $R_6$  is alkyl, in which case  $R_{6'}$  is hydrogen or the same alkyl as  $R_6$ ;

(h)  $R_6$  and  $R_{6'}$  can be taken together as a double bond to  $C_5$  and can be O, S,  $NQ_6$ , or C which can be substituted with one or two groups  $R_5$ , and where  $Q_6$  is alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, heteroaroyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, or heteroaralkylsulfonyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S;

(i)  $R_7$  is hydrogen unless  $R_5$  is alkyl and  $R_{5'}$  is hydrogen, in which case  $R_7$  is the same alkyl as  $R_5$ ;

(2) L is a hydrocarbonyl moiety of 1 to 6 carbon atoms that can be cyclic, with the hydrocarbonyl moiety being optionally substituted with one or more substituents selected from the group consisting of lower alkyl, amino, hydroxy, lower alkoxy, lower alkylamino, lower alkylthio, and oxo; and

(3) B is  $-OZ$  or  $N(Y_1)-D$ , where Z is hydrogen, alkyl, aryl, heteroaryl, cycloalkyl, aralkyl, or heteroaralkyl, D is a moiety that promotes absorption of the derivative or analogue, and  $Y_1$  is hydrogen, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, which, when taken with D, can form a cyclic 5- or 6-membered saturated structure which can contain one other heteroatom which can be O, N, or S, of which N can be further substituted with  $Y_2$ , where  $Y_2$  is alkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, heteroaralkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, aralkoxycarbonyl, heteroaralkoxycarbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl, aralkylaminocarbonyl, or heteroaralkylaminocarbonyl, in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S.

**[0009]** B is either: (i) a moiety with the structure  $-OZ$ , where Z is hydrogen, alkyl, aryl, heteroaryl, cycloalkyl, aralkyl, or heteroaralkyl; or (ii) a moiety with the structure  $-N(Y_1)-D$ , where D is a moiety that promotes absorption of the derivative or analogue that can be substituted as indicated above.

**[0010]** If B is a moiety with the structure -OZ, it is a carboxylic acid or a carboxylic acid ester. Typically, if B is a carboxylic acid ester, the moiety Z is one of methyl, ethyl, propyl, butyl, or isobutyl. More typically, Z is hydrogen or ethyl.

**[0011]** If B is a moiety with the structure -N(Y<sub>1</sub>)-D, Y<sub>1</sub> is hydrogen, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, which, when taken with D, can form a cyclic 5- or 6-membered saturated ring which can contain one other heteroatom which can be O, N, or S, of which N can be further substituted with Y<sub>2</sub>, where Y<sub>2</sub> is alkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, heteroaralkylsulfonyl, alkoxy carbonyl, aryloxy carbonyl, heteroaryloxy carbonyl, aralkoxy carbonyl, heteroaralkoxy carbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroaralkylaminocarbonyl, aralkylaminocarbonyl, heteroaralkylaminocarbonyl, in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S. Typically, Y<sub>1</sub> is hydrogen or lower alkyl. Most typically, Y<sub>1</sub> is hydrogen.

**[0012]** Typically, the tetrahydroinolone derivative or analogue has a logP of from about 1 to about 4 to enhance bioavailability and central nervous system (CNS) penetration. Using this guideline, one of ordinary skill in the art can choose the appropriate moieties B for a particular moiety A in order to ensure the bioavailability and CNS penetration of a tetrahydroinolone analogue or derivative according to the present invention. For example, if a highly hydrophobic moiety A is chosen, with particularly hydrophobic substituents on the tetrahydroinolone moiety, then a more hydrophilic moiety B can be used.

**[0013]** Typically, A is a tetrahydroindolone moiety.

**[0014]** In one alternative, B is a moiety containing at least one carboxyl, carboxamide, carboxyl ester, or carbonyl function.

**[0015]** In another alternative, B is a cyclic or acyclic moiety containing at least one hydroxyl, primary amino, secondary amino, tertiary amino, sulfhydryl, or sulfonamidyl function.

**[0016]** Particular examples of bifunctional conjugates according to the present invention include: (1) 4-[3-(4-oxo-4,5,6,7-tetrahydroindolon-1-yl)propionylamino] benzoic

acid ethyl ester; and 4-[3-(4-oxo-4,5,6,7-tetrahydroindolon-1-yl)propionylamino} benzoic acid.

**[0017]** Another aspect of the present invention is methods of use of the tetrahydroindolone derivatives and analogues described above. One aspect of a method of use of tetrahydroindolone derivatives and analogues according to the present invention is a method of stimulating neuronal function such as improved cognition, involving neuronal regeneration or axo-dendritic complexity in the central and peripheral nervous systems comprising the step of administering an effective amount of a tetrahydroindolone derivative or analogue according to the present invention to the mammal. Another aspect of a method of use of tetrahydroindolone derivatives and analogues according to the present invention is a method of stimulating neuronal function such as improved cognition, involving by initiating neurogenesis in the central nervous system of a mammal comprising the step of administering an effective amount of a tetrahydroindolone derivative or analogue according to the present invention to the mammal. Yet another aspect of a method of use of tetrahydroindolone derivatives and analogues according to the present invention is a method of stimulating neuronal function involving mechanism associated with neuroprotection in the central or peripheral nervous system of a mammal comprising the step of administering an effective amount of a tetrahydroindolone derivative or analogue according to the present invention to the mammal.

**[0018]** Other methods according to the present invention include a method of stimulating neuronal function involving either inhibition of the formation of the amyloid beta-peptide (A $\beta$ ) or stimulating the formation of the secreted derivative of the amyloid precursor protein known as sAPP $\alpha$  by administering to a patient with a neurological disease or a patient at risk of developing a neurological disease an effective quantity of a tetrahydroindolone derivative or analogue according to the present invention.

**[0019]** Another aspect of the present invention is pharmaceutical compositions. A pharmaceutical composition according to the present invention comprises: (1) an effective amount of a tetrahydroindolone derivative or analogue according to the present invention; and (2) a pharmaceutically acceptable carrier.



**BRIEF DESCRIPTION OF THE DRAWINGS**

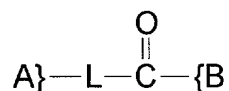
[0020] The following invention will become better understood with reference to the specification, appended claims, and accompanying drawings where:

[0021] Figure 1 is a table (Table 1) depicting a number of the tetrahydroindolone derivatives or analogues according to the present invention together with the minimum effective dose in the passive avoidance test for nootropic activity in mice with intraperitoneal administration.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS****[0022] I. TETRAHYDROINDOLONE DERIVATIVES AND ANALOGUES**

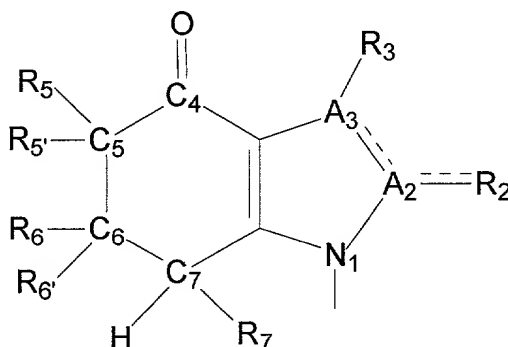
[0023] One aspect of the present invention is tetrahydroindolone derivatives and analogues.

[0024] In its most general aspect, a bifunctional conjugate according to the present invention has the schematic structure:



where:

(1) A is a 9-atom bicyclic moiety in which the five-membered ring has 1 to 3 nitrogen atoms, the bicyclic moiety having the structure:



where:

- (a) N<sub>1</sub> is bonded to L;
- (b) A<sub>2</sub> and A<sub>3</sub> are C or N:

(i) if  $A_2$  and  $A_3$  are both C and the bond between  $A_2$  and  $A_3$  is a single bond, then the bond between  $A_2$  and  $R_2$  is two single bonds to two hydrogen atoms or is a double bond in which  $R_2$  is O or S and  $R_3$  is two hydrogen atoms;

(ii) if  $A_2$  and  $A_3$  are both C and the bond between  $A_2$  and  $A_3$  is a double bond, then  $R_3$  is hydrogen, the bond between  $A_2$  and  $R_2$  is a single bond and  $R_2$  is hydrogen, halo, alkyl, alkenyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, or heteroaralkenyl;

(iii) if  $A_2$  and  $A_3$  are both N, then the bond between  $A_2$  and  $A_3$  is a double bond and  $R_2$  and  $R_3$  are not present;

(iv) if  $A_2$  is N and  $A_3$  is C, then the bond between  $A_2$  and  $A_3$  is a double bond,  $R_2$  is not present, and  $R_3$  is hydrogen;

(v) if  $A_2$  is C,  $A_3$  is N, and the bond between  $A_2$  and  $A_3$  is a double bond, then  $R_3$  is not present, the bond between  $A_2$  and  $R_2$  is a single bond, and  $R_2$  is hydrogen, halo, alkyl, alkenyl, aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, or heteroaralkenyl;

(vi) if  $A_2$  is C,  $A_3$  is N, and the bond between  $A_2$  and  $A_3$  is a single bond, then  $R_3$  is hydrogen, alkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl, the bond between  $A_2$  and  $R_2$  is a double bond, and  $A_2$  is O or S;

(c)  $R_5$  is hydrogen, alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl,  $NH_2$ ,  $NHQ_1$ ,  $NQ_1Q_2$ , OH,  $OQ_1$ , or  $SQ_1$ , where  $Q_1$  and  $Q_2$  are alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, heteroaroyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, or heteroaralkylsulfonyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S, and when  $Q_1$  and  $Q_2$  are present together and are alkyl, they can be taken together to form a 5 or 6 member ring which may contain 1 other heteroatom which can be N, O, or S, of which the N may be further substituted with  $Y_2$ , where  $Y_2$  is alkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, heteroaralkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, aralkoxycarbonyl,

heteroaralkoxycarbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl, aralkylaminocarbonyl, or heteroaralkylaminocarbonyl, in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S;

(d)  $R_{5'}$  is hydrogen unless  $R_5$  is alkyl, in which case  $R_{5'}$  is hydrogen or the same alkyl as  $R_5$ ;

(e)  $R_5$  and  $R_{5'}$  can be taken together as a double bond to  $C_5$  and can be O, S,  $NQ_3$ , or C which can be substituted with one or two groups  $R_5$ , where  $Q_3$  is alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, or heteroaroyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S;

(f)  $R_6$  is hydrogen, alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl,  $NH_2$ ,  $NHQ_4$ ,  $NQ_4Q_5$ , OH,  $OQ_4$ , or  $SQ_4$ , where  $Q_4$  and  $Q_5$  are alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, heteroaroyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, or heteroaralkylsulfonyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S, and when  $Q_1$  and  $Q_2$  are present together and are alkyl, they can be taken together to form a 5 or 6 member ring which may contain 1 other heteroatom which can be N, O, or S, of which the N may be further substituted with  $Y_2$ , where  $Y_2$  is alkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, heteroaralkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, aralkoxycarbonyl, heteroaralkoxycarbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl, aralkylaminocarbonyl, or heteroaralkylaminocarbonyl, in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S;

(g)  $R_{6'}$  is hydrogen unless  $R_6$  is alkyl, in which case  $R_{6'}$  is hydrogen or the same alkyl as  $R_6$ ;

(h)  $R_6$  and  $R_{6'}$  can be taken together as a double bond to  $C_5$  and can be O, S,  $NQ_6$ , or C which can be substituted with one or two groups  $R_5$ , and where

Q6 is alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, heteroaroyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, or heteroaralkylsulfonyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S;

(i) R<sub>7</sub> is hydrogen unless R<sub>5</sub> is alkyl and R<sub>5'</sub> is hydrogen, in which case R<sub>7</sub> is the same alkyl as R<sub>5</sub>;

(2) L is a hydrocarbonyl moiety of 1 to 6 carbon atoms that can be cyclic, with the hydrocarbonyl moiety being optionally substituted with one or more substituents selected from the group consisting of lower alkyl, amino, hydroxy, lower alkoxy, lower alkylamino, lower alkylthio, and oxo; and

(3) B is -OZ or N(Y<sub>1</sub>)-D, where Z is hydrogen, alkyl, aryl, heteroaryl, cycloalkyl, aralkyl, or heteroaralkyl, D is a moiety that promotes absorption of the derivative or analogue, and Y<sub>1</sub> is hydrogen, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, which, when taken with D, can form a cyclic 5- or 6-membered saturated structure which can contain one other heteroatom which can be O, N, or S, of which N can be further substituted with Y<sub>2</sub>, where Y<sub>2</sub> is alkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, heteroaralkylsulfonyl, alkoxycarbonyl, aryloxy carbonyl, heteroaryloxy carbonyl, aralkoxy carbonyl, heteroaralkoxy carbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl, aralkylaminocarbonyl, or heteroaralkylaminocarbonyl, in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S.

**[0025]** As shown in Formula (I), the moiety A has a six-membered ring fused to a five-membered ring, as in a tetrahydroindolone moiety. The five-membered ring can have one, two, or three nitrogen atoms as indicated, but always has a nitrogen atom at the position that would correspond to the 9-position of a purine moiety with which the tetrahydroindolone moiety is isosteric. Typically, the five-membered ring has one nitrogen atom as in tetrahydroindolone. This nitrogen atom is covalently bonded to the linker L.

**[0026]** Typically A is a tetrahydroindolone moiety in which A<sub>7</sub> is carbon and A<sub>8</sub> is carbon. The tetrahydroindolone moiety can be variously substituted.

**[0027]** Typically, Y<sub>1</sub> is hydrogen or lower alkyl. Most typically, Y<sub>1</sub> is hydrogen.

**[0028]** In many examples, in a tetrahydroindolone analogue or derivative according to the present invention, the moiety B has a biological, physiological, or pharmacological function, and the tetrahydroindolone analogue or derivative is referred to as a “bifunctional conjugate.” However, it is not required in tetrahydroindolone analogues or derivatives according to the present invention that the moiety B have a biological, physiological, or pharmacological function. The moiety B can serve as a carrier to improve bioavailability or to optimize the physical characteristics of the molecule without having a separate biological, physiological function, or pharmacological function.

**[0029]** In many tetrahydroindolone analogues or derivatives according to the present invention, the moiety B includes a *p*-aminobenzoic acid, or a *p*-aminobenzoic acid ester. However, the moiety B can include other groups.

**[0030]** Typically, the tetrahydroindolone derivative or analogue has a logP of from about 1 to about 4 to enhance bioavailability and central nervous system (CNS) penetration. Using this guideline, one of ordinary skill in the art can choose the appropriate moieties B for a particular moiety A in order to ensure the bioavailability and CNS penetration of a tetrahydroindolone analogue or derivative according to the present invention. For example, if a highly hydrophobic moiety A is chosen, with particularly hydrophobic substituents on the tetrahydroindolone moiety, then a more hydrophilic moiety B can be used.

**[0031]** The linker L is described further below. L is a hydrocarbyl moiety of 1 to 6 carbon atoms that can be cyclic, with the hydrocarbyl moiety being optionally substituted with one or more substituents selected from the group consisting of lower alkyl, amino, hydroxy, lower alkoxy, lower alkylamino, lower alkylthio, and oxo.

**[0032]** In accordance with the present invention, and as used herein, the following terms, when appearing alone or as part of a moiety including other atoms or groups, are defined with the following meanings, unless explicitly stated otherwise. In addition, all groups described herein can be optionally substituted unless such substitution is

excluded. The term "alkyl," as used herein at all occurrences, refers to saturated aliphatic groups including straight-chain, branched-chain, and cyclic groups, all of which can be optionally substituted. Preferred alkyl groups contain 1 to 10 carbon atoms. Suitable alkyl groups include methyl, ethyl, and the like, and can be optionally substituted. The term "alkenyl," as used herein at all occurrences, refers to unsaturated groups which contain at least one carbon-carbon double bond and includes straight-chain, branched-chain, and cyclic groups, all of which can be optionally substituted. Preferable alkenyl groups have 2 to 10 carbon atoms. The term "alkoxy" refers to the ether  $\text{—O—alkyl}$ , where alkyl is defined as above. The term "aryl" refers to aromatic groups which have at least one ring having a conjugated  $\pi$ -electron system and includes carbocyclic aryl and biaryl, both of which may be optionally substituted. Preferred aryl groups have 6 to 10 carbon atoms. The term "aralkyl" refers to an alkyl group substituted with an aryl group. Suitable aralkyl groups include benzyl and the like; these groups can be optionally substituted. The term "aralkenyl" refers to an alkenyl group substituted with an aryl group. The term "heteroaryl" refers to carbon-containing 5-14 membered cyclic unsaturated radicals containing one, two, three, or four O, N, or S heteroatoms and having 6, 10, or 14  $\pi$ -electrons delocalized in one or more rings, e.g., pyridine, oxazole, indole, thiazole, isoxazole, pyrazole, pyrrole, each of which can be optionally substituted as discussed above. The term "sulfonyl" refers to the group  $\text{—S(O}_2\text{)—}$ . The term "alkanoyl" refers to the group  $\text{—C(O)R}_g$ , where  $\text{R}_g$  is alkyl. The term "aroyl" refers to the group  $\text{—C(O)R}_g$ , where  $\text{R}_g$  is aryl. Similar compound radicals involving a carbonyl group and other groups are defined by analogy. The term "aminocarbonyl" refers to the group  $\text{—NHC(O)—}$ . The term "oxycarbonyl" refers to the group  $\text{—OC(O)—}$ . The term "heteroaralkyl" refers to an alkyl group substituted with a heteroaryl group. Similarly, the term "heteroaralkenyl" refers to an alkenyl group substituted with a heteroaryl group. Where used herein, the term "lower," in reference to an alkyl or the alkyl portion of another group including alkyl, is defined as a group containing one to ten carbon atoms, more typically one to six carbon atoms. The term "optionally substituted" refers to one or more substituents that are typically lower alkyl, aryl, amino, hydroxy, lower alkoxy, aryloxy, lower alkylamino, arylamino, lower alkylthio, arylthio, or oxo, in some cases, other groups can be included, such as cyano, acetoxy,

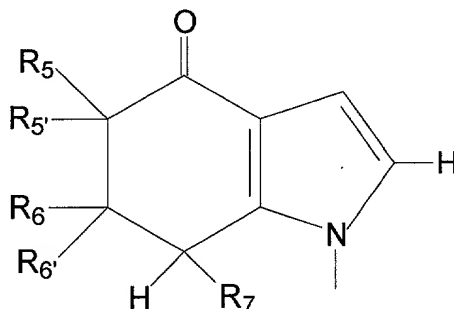
or halo. The term "halo" refers generally to fluoro, chloro, bromo, or iodo; more typically, "halo" refers to chloro.

**[0033]** A preferred linker has the structure  $-(CH_2)_n-$  wherein  $n$  is an integer from 1 to 6. As detailed below, for most preferred embodiments of tetrahydroindolone derivatives and analogues according to the present invention, a preferred linker has  $n$  equal to 2 or 3. The following are particular examples of tetrahydroindolone derivatives and analogues according to the present invention.

**[0034]** A number of tetrahydroindolone derivatives or analogues according to the present invention are optically active, owing to the presence of chiral carbons or other centers of asymmetry. In cases where tetrahydroindolone derivatives or analogues according to the present invention are optically active, all of the possible enantiomers or diastereoisomers are included unless otherwise indicated despite possible differences in activity.

**[0035]** Particularly preferred tetrahydroindolone moieties for the moiety A are described below.

**[0036]** One example of a tetrahydroindolone moiety for the moiety A is a tetrahydroindolone moiety of Formula (II), below, in which:



(1)  $R_5$  is hydrogen, alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl,  $NH_2$ ,  $NHW_1$ ,  $NQ_1Q_2$ , OH,  $OQ_1$ , or  $SQ_1$ , where  $Q_1$  and  $Q_2$  are alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, or heteroaroyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S, and where  $W_1$  is alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, or heteroaroyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl,

aralkylsulfonyl, or heteroaralkylsulfonyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S;

(2)  $R_{5'}$  is hydrogen;

(3)  $R_6$  is hydrogen, alkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl,  $NH_2$ ,  $NHW_1$ ,  $NQ_1Q_2$ , OH,  $OQ_1$ , or  $SQ_1$ , where  $Q_1$  and  $Q_2$  are alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, or heteroaroyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S, and where  $W_1$  is alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, or heteroaroyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, or heteroaralkylsulfonyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S;

(4)  $R_{6'}$  is hydrogen; and

(5)  $R_7$  is hydrogen.

**[0037]** In one particularly preferred embodiment,  $R_5$ ,  $R_{5'}$ ,  $R_6$ ,  $R_{6'}$ , and  $R_7$  are all hydrogen. In this particularly preferred embodiment, the moiety A is an unsubstituted tetrahydroindolone moiety.

**[0038]** In addition to these examples of moieties suitable as moiety A, other moieties can serve as moiety A, including moieties with two or three nitrogen atoms or moieties with substituents at  $R_2$ .

**[0039]** In general, tetrahydroindolone derivatives and analogues that are within the scope of the present invention also include salts and prodrug esters of these bifunctional conjugates. It is well known that organic compounds, including substituted tetrahydroindolones and other components of these tetrahydroindolone derivatives and analogues, have multiple groups that can accept or donate protons, depending upon the pH of the solution in which they are present. These groups include carboxyl groups, hydroxyl groups, amino groups, sulfonic acid groups, and other groups known to be involved in acid-base reactions. The recitation of a tetrahydroindolone derivative or



analogue includes such salt forms as occur at physiological pH or at the pH of a pharmaceutical composition unless specifically excluded.

**[0040]** Similarly, prodrug esters can be formed by reaction of either a carboxyl or a hydroxyl group on the tetrahydroindolone derivative or analogue with either an acid or an alcohol to form an ester. Typically, the acid or alcohol includes a lower alkyl group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tertiary butyl. These groups can be substituted with substituents such as hydroxy, , or other substituents. Such prodrugs are well known in the art and need not be described further here. The prodrug is converted into the active compound by hydrolysis of the ester linkage, typically by intracellular enzymes. Other suitable groups that can be used to form prodrug esters are well known in the art.

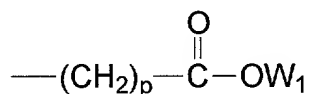
**[0041]** As indicated above, the linker L is a hydrocarbyl moiety of one to six carbon atoms, with the hydrocarbyl moiety being optionally substituted with one or more substituents selected from the group consisting of lower alkyl, amino, hydroxy, lower alkoxy, lower alkylamino, lower alkythio, and oxo. Preferably, the linker L has the structure  $-(CH_2)_n-$  wherein n is an integer from 1 to 6. As detailed below, for most preferred embodiments of tetrahydroindolone derivatives and analogues according to the present invention, a preferred linker has n equal to 2 or 3.

**[0042]** The moiety B is either: (i)  $-OZ$ , where Z is hydrogen, alkyl, aryl, heteroaryl, cycloalkyl, aralkyl, or heteroaralkyl ; or (ii)  $N(Y_1)-D$ , where D is a moiety that promotes absorption of the derivative or analogue, and  $Y_1$  is hydrogen, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, which, when taken with D, can form a cyclic 5- or 6-membered saturated ring which can contain one other heteroatom which can be O, N, or S, of which N can be further substituted with  $Y_2$ , where  $Y_2$  is alkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, heteroaralkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, aralkoxycarbonyl, heteroaralkoxycarbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl, aralkylaminocarbonyl, heteroaralkylaminocarbonyl, in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S. Typically,  $Y_1$  is hydrogen. Where the moiety B is  $-OZ$ , the moiety B is a

carboxylic acid or carboxylic acid or ester. Typically, where B is a carboxylic acid ester, the moiety Z is a lower alkyl, such as methyl, ethyl, butyl, propyl, or isopropyl.

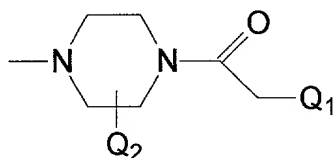
**[0043]** In one alternative, the moiety D, as described above, is a moiety having at least one polar, charged, or hydrogen-bond-forming group to improve the metabolic and bioavailability properties of the tetrahydroindolone derivative or analogue. The moiety D can be, but is not limited to, a moiety with physiological or biological activity such as nootropic activity. In one alternative, the moiety D can be a moiety containing at least one carboxyl, carboxamide, carboxyl ester, or carbonyl function. In another alternative, the moiety D can be a moiety containing at least one hydroxyl, primary amino, secondary amino, tertiary amino, sulfhydryl, or sulfonamidyl function. The moiety D can be cyclic or acyclic. Preferred examples of the moiety D are described below.

**[0044]** When the moiety D is a cyclic or acyclic moiety containing at least one carbonyl, carboxamide, carboxyl ester, or carbonyl function, in one preferred example, D is a carboxylic acid or carboxylic acid ester with the structure



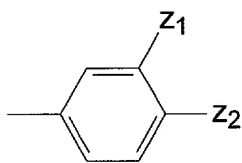
wherein p is an integer from 1 to 6 and W<sub>1</sub> is selected from the group consisting of hydrogen and lower alkyl. Typically, if W<sub>1</sub> is lower alkyl, it is methyl, ethyl, propyl, butyl, or isobutyl. Typically, p is 3. Typically, W<sub>1</sub> is hydrogen or ethyl.

**[0045]** In another preferred example, D and Y<sub>1</sub> are taken together to form a piperazine derivative as described in D. Manetti et al., "Molecular Simplification of 1,4-Diazabicyclo[4.3.0]nonan-9-ones Gives Piperazine Derivatives That Maintain High Nootropic Activity," J. Med. Chem. 43: 4499-4507 ("Manetti et al. (2000) (II)"). B is an analogue of structure



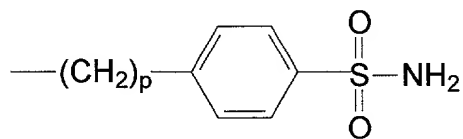
wherein Q<sub>1</sub> is hydrogen, methyl, ethyl, butyl, or propyl, Q<sub>2</sub> is hydrogen or methyl, where, if Q<sub>2</sub> is methyl, it can be located at either of the two possible positions in the piperazine ring..

**[0046]** In another preferred example, D has the structure



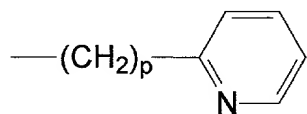
where one of Z<sub>1</sub> and Z<sub>2</sub> is hydrogen, and the other of Z<sub>1</sub> and Z<sub>2</sub> is –COOH or –COOW<sub>1</sub>, wherein W<sub>1</sub> is alkyl. Typically, W<sub>1</sub> is selected from the group consisting of methyl, ethyl, propyl, butyl, and isobutyl. Either of Z<sub>1</sub> or Z<sub>2</sub> can be hydrogen. When Z<sub>1</sub> is hydrogen and Z<sub>2</sub> is –COOH, the moiety B is *p*-aminobenzoic acid (PABA). When Z<sub>1</sub> is –COOH and Z<sub>2</sub> is hydrogen, the moiety B is *m*-aminobenzoic acid (MABA). When Z<sub>1</sub> is hydrogen and Z<sub>2</sub> is –COOW<sub>1</sub>, the moiety B is an ester of *p*-aminobenzoic acid (PABA). When Z<sub>1</sub> is –COOW<sub>1</sub> and Z<sub>2</sub> is hydrogen, the moiety B is an ester of *m*-aminobenzoic acid (MABA). Typically, these esters are ethyl esters.

**[0047]** When the moiety D is a moiety that contains at least one hydroxyl, primary amino, secondary amino, tertiary amino, sulfhydryl, or sulfonamidyl function, in one preferred example, D is a phenylsulfonamidyl moiety of structure



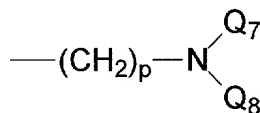
wherein p is an integer from 0 to 6. Typically, p is 2.

**[0048]** In another preferred example, D is an alkylpyridyl moiety of structure



wherein p is an integer from 1 to 6. Typically, p is 1.

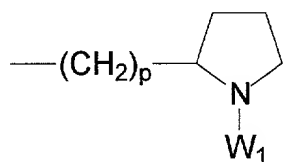
**[0049]** In another preferred example, D is a dialkylaminoalkyl moiety of the structure



wherein p is an integer from 1 to 6 and Q<sub>7</sub> and Q<sub>8</sub> are alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, or heteroaroyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be

N, O, or S, and when Q<sub>7</sub> and Q<sub>8</sub> are present together and are alkyl, they can be taken together to form a 5 or 6 member ring which may contain 1 other heteroatom which can be N, O, or S, of which the N may be further substituted with Y<sub>2</sub>, where Y<sub>2</sub> is alkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, heteroaralkylsulfonyl, alkoxy carbonyl, aryloxy carbonyl, heteroaryloxy carbonyl, aralkoxy carbonyl, heteroaralkoxy carbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl, aralkylaminocarbonyl, or heteroaralkylaminocarbonyl, in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S, and where W<sub>1</sub> is alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, alkanoyl, aroyl, aralkanoyl, heteroaralkanoyl, or heteroaroyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, or heteroaralkylsulfonyl in which the alkyl portions can be cyclic and can contain from 1 to 3 heteroatoms which can be N, O, or S. Where Q<sub>7</sub> and Q<sub>8</sub> can be taken together to form a five or six member optionally substituted ring, the ring is typically pyrrolidine, piperidine, or morpholine. The pyrrolidine ring can be optionally substituted with oxo. The piperidine ring can be optionally substituted with methyl or ethyl. Typically, p is 2 or 3.

**[0050]** In another preferred example, D is an alkylpyrrolidine moiety of the structure



wherein p is an integer from 1 to 6 and W<sub>1</sub> is selected from the group consisting of methyl, ethyl, and propyl. Typically, W<sub>1</sub> is methyl. Typically, p is 2.

**[0051]** Preferably, a tetrahydroindolone analogue or derivative according to the present invention has a logP of from about 1 to about 4 in order to optimize bioavailability and CNS penetration of the tetrahydroindolone analogue or derivative.

**[0052]** In general, any moiety A can be combined with any linker L and any moiety B, including the appropriate moiety D, to produce a tetrahydroindolone analogue or derivative according to the present invention. However, there exist a number of

particularly preferred tetrahydroindolone analogues or derivatives according to the present invention. These include the following:

(1) 4-[3-(4-oxo-4,5,6,7-tetrahydroindolon-1-yl)propionylamino} benzoic acid ethyl ester (Example 1); and

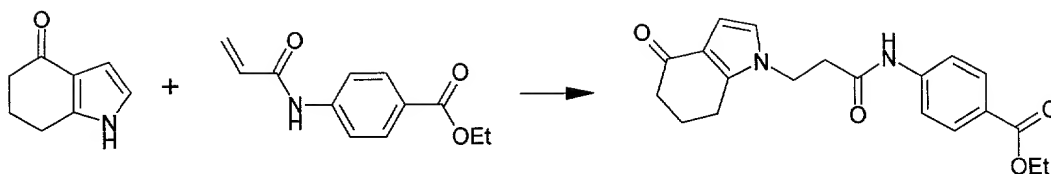
(2) 4-[3-(4-oxo-4,5,6,7-tetrahydroindolon-1-yl)propionylamino} benzoic acid.

## **[0053]** II. METHODS OF SYNTHESIS OF TETRAHYDROINDOLONE DERIVATIVES AND ANALOGUES ACCORDING TO THE PRESENT INVENTION

**[0054]** Methods for synthesis of tetrahydroindolone derivatives and analogues according to the present invention are modified from those described, for example, in U.S. Patent No. 5,091,432 to Glasky, incorporated herein by this reference. In one general route in which the tetrahydroindolone derivatives and analogues according to the present invention contain a tetrahydroindolone moiety, the tetrahydroindolone moiety is substituted with a linker which in turn is linked to the moiety B that completes the molecule as described above. This route comprises the steps of: (1) synthesizing an appropriately substituted tetrahydroindolone moiety linked to an aliphatic linker in which the linker is terminated with a carboxyl group protected such as with an alkyl ester; (2) hydrolyzing the alkyl ester (or other analogous protecting group) to yield a carboxylic acid; (3) activating the free carboxylic acid by converting it to a nitrophenyl ester; (4) reacting the nitrophenyl ester with an appropriate group that can form an amide or other stable covalent linkage with the carboxyl moiety, with appropriate protection for the moiety reacting with the ester if required; and (5) hydrolyzing the protective group protecting the moiety reacting with the ester to produce the final product.

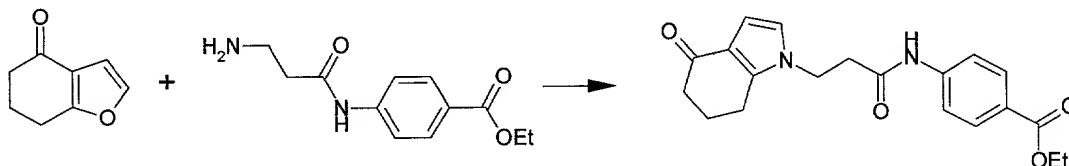
**[0055]** The length of the aliphatic linker covalently bound to the tetrahydroindolone moiety can be varied to vary the distance between the tetrahydroindolone moiety and the moiety B in the tetrahydroindolone derivative or analogue.

**[0056]** Other methods for functionalization of the tetrahydroindolone moiety include the use of Michael addition of the 1-nitrogen of the tetrahydroindolone moiety to the terminal methylene of an acrylamide which already contains the moiety B. (see below)



**[0057]** Another reaction that may be used to functionalize tetrahydroindolones is the Mitsunobu reaction. The Mitsunobu reaction is a highly versatile method for the introduction of widely varying functionality upon the tetrahydroindolone moiety, because of the wide assortment of primary alcohols that are commercially available for use in this reaction.

**[0058]** Yet another method of synthesis of tetrahydroindolone derivatives or analogues is via an appropriately substituted tetrahydrobenzofuranone. The tetrahydrobenzofuranone is reacted with a primary amine attached to the appropriate linker and B moiety (see below). Matsumoto, *et al.* "A Facile Synthesis of 4-Oxo-4,5,6,7-tetrahydroindolone." *Heterocycles* 22: 2313-2316 (1984)



### **[0059]** III. METHODS OF USE OF TETRAHYDROINDOLONE DERIVATIVES AND ANALOGUES ACCORDING TO THE PRESENT INVENTION

**[0060]** One aspect of a method of use of tetrahydroindolone derivatives and analogues according to the present invention is a method of stimulating regeneration of a mammalian neuron in the peripheral nervous system of a mammal comprising the step of administering an effective amount of a tetrahydroindolone derivative or analogue according to the present invention to the mammal.

**[0061]** Another aspect of a method of use of tetrahydroindolone derivatives and analogues according to the present invention is a method of stimulating neurogenesis in the central nervous system of a mammal comprising the step of administering an effective amount of a tetrahydroindolone derivative or analogue according to the present invention to the mammal.

**[0062]** Yet another aspect of a method of use of tetrahydroindolone derivatives and analogues according to the present invention is a method of stimulating neuroprotection in the central or peripheral nervous system of a mammal comprising the step of administering an effective amount of a tetrahydroindolone derivative or analogue according to the present invention to the mammal.

**[0063]** Exemplary dosages in accordance with the teachings of the present invention for these tetrahydroindolone derivatives and analogues range from 0.0001 mg/kg to 60 mg/kg, though alternative dosages are contemplated as being within the scope of the present invention. Suitable dosages can be chosen by the treating physician by taking into account such factors as the size, weight, age, and sex of the patient, the physiological state of the patient, the severity of the condition for which the tetrahydroindolone derivative or analogue is being administered, the response to treatment, the type and quantity of other medications being given to the patient that might interact with the tetrahydroindolone derivative or analogue, either potentiating it or inhibiting it, and other pharmacokinetic considerations such as liver and kidney function.

**[0064]** The administration of tetrahydroindolone derivatives or analogues according to the present invention is believed to increase the level of mRNA encoding at least one neurotrophic factor in a tissue that is in chemical communication with a motor neuron or a sensory neuron. The term "chemical communication" as used herein means that at least one chemical substance that can affect the growth, differentiation, survival, or functioning of motor neurons or sensory neurons can flow from the tissue to the neuron. The neurotrophic factor stimulates the growth of neurons.

**[0065]** The term "effective amount" as used herein in this specification means an amount of the tetrahydroindolone derivative or analogue that causes a detectable increase in the messenger RNA level of at least one of the recited neurotrophic factors or of another neurotrophic factor known in the art that can be measured. Methods of measuring the mRNA levels typically involve hybridization to probes containing mRNA-specific sequences and detecting the quantity of hybrid nucleic acid formed. The hybrid nucleic acid formed is typically detected by a label incorporated in one of the two nucleic acid strands forming the hybrid. This label can be radioactive or non-radioactive; if non-radioactive, it can be fluorescent, chemiluminescent, bioluminescent, enzymatic, or can

make use of another detectable property. Detection can also be performed using the polymerase chain reaction (PCR) mechanism or a variant thereof. PCR is described in detail in U.S. Patent No. 4,683,195 to Mullis et al. and U.S. Patent No. 4,683,202 to Mullis et al. Other detection methods, including other amplification methods, are known in the art. One particularly suitable method uses reverse transcription with MMLV reverse transcriptase followed by PCR.

**[0066]** Another method employing tetrahydroindolone derivatives and analogues according to the present invention is a method of either increasing neuronal function inhibiting the formation of the amyloid beta-peptide ( $A\beta$ ) or stimulating the formation of the secreted derivative of the amyloid precursor protein known as sAPP $\alpha$  by administering to a patient with a neurological disease or a patient at risk of developing a neurological disease an effective quantity of a tetrahydroindolone derivative or analogue according to the present invention as described above. The neurological disease can be a neurodegenerative disease, such as, but not limited to, Alzheimer's disease (AD). Alternatively, the neurological disease can be a neurodevelopmental disorder such as, but not limited to, Down's Syndrome.

**[0067]** Another aspect of methods according to the present invention is a method of treating peripheral neuropathy comprising administering to a patient with peripheral neuropathy an effective quantity of a tetrahydroindolone derivative or analogue according to the present invention. Typically, in this method, the administration of the tetrahydroindolone derivative or analogue induces peripheral nerve sprouting in the skin of the patient to whom the tetrahydroindolone derivative or analogue is administered. The peripheral nerve sprouting can be nociceptive nerve sprouting. Typically, the nociceptive nerve sprouting is induced without the occurrence of hyperalgesia. The peripheral neuropathy can be diabetic neuropathy or can be a neuropathy associated with the following conditions: acromegaly, hypothyroidism, AIDS, leprosy, Lyme disease, systemic lupus erythematosus, rheumatoid arthritis, Sjögren's Syndrome, periarteritis nodosa, Wegener's granulomatosis, cranial arteritis, sarcoidosis or the administration of other therapeutic agents, e.g. oncolytic drugs. Yet another aspect of the present invention is a method of increasing neuronal function by inducing proliferation and differentiation of neuronal precursor cells. In general, the method



comprises administering to a mammal an effective quantity of a tetrahydroindolone derivative or analogue according to the present invention as described above to induce proliferation of neuronal precursor cells in the mammal.

**[0068]** Depending upon the particular needs of the individual subject involved, the tetrahydroindolone derivative or analogue according to the present invention may be administered in various doses to provide effective treatment concentrations based upon the teachings of the present invention. What constitutes an effective amount of the selected tetrahydroindolone derivative or analogue will vary based upon such factors as the activity of the selected tetrahydroindolone derivative or analogue, the physiological characteristics of the subject, the extent or nature of the subject's disease or condition, and the method of administration. Generally, initial doses will be modified to determine the optimum dosage for treatment of the particular mammalian subject. The tetrahydroindolone derivatives or analogues can be administered using a number of different routes including orally, topically, transdermally, intraperitoneal injection, or intravenous injection directly into the bloodstream. Of course, effective amounts of the tetrahydroindolone derivative or analogue can also be administered through injection into the cerebrospinal fluid or infusion directly into the brain, if desired.

**[0069]** The methods of the present invention can be effected using a tetrahydroindolone derivative or analogue according to the present invention administered to a mammalian subject either alone or in combination as a pharmaceutical formulation. Further, the tetrahydroindolone derivative or analogue according to the present invention can be combined with pharmaceutically acceptable excipients and carrier materials such as inert solid diluents, aqueous solutions, or non-toxic organic solvents. If desired, these pharmaceutical formulations can also contain preservatives and stabilizing agents and the like, as well as minor amounts of auxiliary substances such as wetting or emulsifying agents, as well as pH buffering agents and the like which enhance the effectiveness of the active ingredient. The pharmaceutically acceptable carrier can be chosen from those generally known in the art including, but not limited to, human serum albumin, ion exchangers, dextrose, alumina, lecithin, buffer substances such as phosphate, glycine, sorbic acid, propylene glycol, polyethylene

glycol, and salts or electrolytes such as protamine sulfate, sodium chloride, or potassium chloride. Other carriers can be used.

**[0070]** Liquid compositions can also contain liquid phases either in addition to or to the exclusion of water. Examples of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, organic esters such as ethyl oleate, and water-oil emulsions.

**[0071]** The compositions can be made into aerosol formations (i.e., they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichloromethane, propane, or nitrogen. Other suitable propellants are known in the art.

**[0072]** Formulations suitable for parenteral administration, such as, for example, by intravenous, intramuscular, intradermal, and subcutaneous routes, include aqueous and non-aqueous isotonic sterile injection solutions. These can contain antioxidants, buffers, preservatives, bacteriostatic agents, and solutes that render the formulation isotonic with the blood of the particular recipient. Alternatively, these formulations can be aqueous or non-aqueous sterile suspensions that can include suspending agents, thickening agents, solublizers, stabilizers, and preservatives. Compositions suitable for use in methods according to the present invention can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically, or intrathecally. Formulations of tetrahydroindolone derivatives or analogues suitable for use in methods according to the present invention can be presented in unit-dose or multi-dose sealed containers, in physical forms such as ampules or vials.

**[0073]** IV. PHARMACEUTICAL COMPOSITIONS

**[0074]** Another aspect of the present invention is pharmaceutical compositions. A pharmaceutical composition according to the present invention comprises: (1) an effective amount of a tetrahydroindolone derivative or analogue according to the present invention as described above; and (2) a pharmaceutically acceptable carrier.

**[0075]** A pharmaceutically acceptable carrier can be chosen from those generally known in the art including, but not limited to, human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as potassium sulfate. Other carriers can be used.

**[0076]** The invention is illustrated by the following Examples. These Examples are presented for illustrative purposes only and are not intended to limit the invention.

**[0077]** Example 1

**[0078]** Synthesis of 4-[3-(4-Oxo-4,5,6,7-tetrahydroindolon-1-yl)propionylamino} Benzoic Acid Ethyl Ester

**[0079]** To a solution of acryloyl chloride (1.08 mL, 13.32 mmol) in dry dichloromethane (10 mL) was slowly added dropwise, via addition funnel, a solution of 4-aminobenzoic acid ethyl ester (2.0 g, 12.11 mmol) and triethylamine (1.86 mL, 13.32 mmol) in dry dichloromethane (5 mL) over about 30 minutes. The reaction was stirred at room temperature for 16 hours. The solution was then partitioned between dichloromethane and water in a separatory funnel. The organic layer was washed with 2 N HCl, dried with sodium sulfate, and concentrated in vacuo to dryness. The resulting residue was triturated with hexane, and vacuum filtered to yield 2.02 g (76%) of 4-acryloylaminobenzoic acid ethyl ester as a solid.

**[0080]** In the second step, 4-acryloylaminobenzoic acid ethyl ester (200 mg, 0.91 mmol), 1,5,6,7-tetrahydro-4H-indol-4-one (123 mg, 0.91 mmol) and potassium carbonate (126 mg, 0.91 mmol) were combined in dry N,N-dimethylformamide (3 mL) and stirred at room temperature for 24 hours, then heated to 60 C for 6 hours. Water (20 mL) was added and the resulting precipitate was vacuum filtered washing with water. The isolated solid was dried under vacuum to yield 180 mg (56%) of the title compound.

**[0081]** Example 2

**[0082]** Passive Avoidance Method of Testing Compounds

**[0083]** Passive avoidance is an acute memory paradigm that indicates an increased neocortical function. In this paradigm mice are allowed to enter a dark compartment from a lighted compartment, but are given a footshock (2 mA for 5 seconds) when they enter the dark compartment. Twenty-four hours after this training session, animals that are placed back in the lighted compartment of two compartment (light-dark) apparatus do not make the transition into the dark compartment. If an amnesic agent (30 mg/kg cycloheximide i.p. in saline) immediately after the training session is given to the

animals, they will make the transition into the dark compartment (i.e. memory of the shock is lost). Compounds with suspected nootropic or anti-amnestic effects are given by i.p. administration two hours prior to the training trial in attempt to block the effects of cycloheximide. Mice that exhibit positive nootropic effects are those that avoid moving into the dark chamber. This behavioral response is defined as passive avoidance. A no effect response in this test is defined as a failure to stay in the lighted compartment for 120 seconds.

**[0084]** Example 3

**[0085]** Passive Avoidance Test of 4-[3-(4-Oxo-4,5,6,7-tetrahydroindolon-1-yl)propionylamino} Benzoic Acid Ethyl Ester (Example 1) Administered Intraperitoneally

**[0086]** The passive avoidance test of Example 2 was used to test 4-[3-(4-oxo-4,5,6,7-tetrahydroindolon-1-yl)propionylamino} benzoic acid ethyl ester (Example 1) with intraperitoneal administration with doses ranging from 0.00001 to 10 mg/kg. The minimum effective dose (MED) is 0.001 mg/kg.

**ADVANTAGES OF THE PRESENT INVENTION**

**[0087]** The present invention provides tetrahydroindolone analogues and derivatives that exert a number of biological and physiological functions, particularly increased neuronal function that may involve nerve regeneration in the peripheral nervous system, neurogenesis in the central nervous system, and neuroprotection. The tetrahydroindolone analogues and derivatives of the present invention are capable of passing through the blood-brain barrier and exerting their effects in the central nervous system.. The components of the tetrahydroindolone analogue or derivative can be chosen to optimize the desired activity or range of activities of the molecule and to increase bioavailability.

**[0088]** Although the present invention has been described in considerable detail, with reference to certain preferred versions thereof, other versions and embodiments are possible. Therefore, the scope of the invention is determined by the following claims.